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Comparison of Cartridge-Based Nucleic Acid Amplification Test with Fine Needle Aspiration Findings in Suspected Tubercular Lymphadenitis

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ABSTRACT
Lymphadenopathy is a common but non-specific presentation of tuberculosis. Fine Needle Aspiration Cytology (FNAC) and Ziehl Neelsen (ZN) stain are preferred investigations of choice in evaluating lymphadenopathy. The aspirate obtained from FNAC may be used to perform a cartridge-based nucleic acid amplification test (CBNAAT). This study aimed to assess the efficacy of CBNAAT in the diagnosis of tubercular lymphadenopathy and to compare the CBNAAT and ZN stain microscopy results with FNAC findings. The study was conducted for a year at a tertiary referral center. FNAC was performed on 86 cases suspected of tubercular lymphadenitis. CBNAAT was performed on FNA material obtained from all these cases. Results of CBNAAT and ZN microscopy were compared with FNAC findings. CBNAAT was positive in 29 cases and ZN stain was positive in 15 cases. Out of 73 cases reported as tubercular lymphadenitis on FNAC, one case of reactive lymphoid hyperplasia and suppurative lymphadenitis was positive on the CBNAAT test. The sensitivity, specificity, and positive and negative predictive values of CBNAAT were 39.72%, 84.61%, 93.54%, and 20%, respectively. CBNAAT from FNA material can be used as an adjuvant diagnostic test in suspected tubercular lymphadenitis. However, CBNAAT cannot replace FNAC in the diagnosis of extra-pulmonary tuberculosis.

KEYWORDS: acid-fast bacilli; CBNAAT; FNAC; lymphadenitis; tuberculosis

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1. Introduction

The burden of tuberculosis in India is highest, with an incidence of 21% globally [1]. Tuberculosis is the leading cause of lymphadenopathy in India, and superficial lymphadenopathy is the most common presentation of extra-pulmonary tuberculosis. The World Health Organization (WHO) defines extra-pulmonary tuberculosis as an infection by *Mycobacterium tuberculosis* that affects tissues and organs outside the pulmonary parenchyma [2]. Early diagnosis and treatment are crucial in such cases and save many lives. Conventional diagnostic methods like sputum examination and

chest X-ray are helpful in the diagnosis of pulmonary disease but not valuable for detecting extra-pulmonary tuberculosis and Rifampicin resistance [3].

FNAC is a rapid, simple, and widely used diagnostic test in evaluating lymphadenopathy. Ancillary tests like ZN stain and Auramine and Rhodamine stain on FNA material aid in diagnosis. Both FNAC and smear microscopy cannot identify species and drug resistance. In resource-poor countries like India, mycobacterial culture and drug susceptibility testing are not always available, and their results take four to eight weeks or even longer [4].

To overcome such limitations, reliable and more rapid methods are needed in the diagnostic field. CBNAAT/Gene Xpert MTB/RIF1 (Cepheid, USA) was developed in December 2010 to diagnose tuberculosis. Revised National Tuberculosis Control Programme (RNTCP) adopted CBNAAT in 2012 and started a pilot project in Maharashtra, India [5]. CBNAAT is based on a closed-system Polymerase Chain Reaction (PCR) requiring minimal technical expertise to diagnose tuberculosis and rifampicin resistance, with the results available in two hours [6].

This study was undertaken to assess the role of CBNAAT using FNA material in the diagnosis of tubercular lymphadenopathy and to compare it with cytology and smear microscopy results.

2. Materials and Methods

2.1 Study Design

This research was a retrospective analysis of routinely collected data. Data were collected at a tertiary referral center over 12 months, from June 2020 to May 2021. Approval of the study protocols was received from the Institutional Ethics Committee of the Netaji Subhash Chandra Bose Medical College, Jabalpur, with a reference number IEC/2022/4401.

2.2 Participants

Samples were selected randomly from all the patients coming to the out-patient department (OPD) and those suspected of tubercular lymphadenitis clinico-radiologically. Patients on anti-Koch treatment (AKT) for more than one month were excluded as they were already diagnosed, and probably CBNAAT will give false-negative results because of the low bacterial count. Patients who refused to give consent for both tests were also excluded.

2.3 Procedure

Written consent and assent forms were taken from all participants. Under aseptic precautions, the FNAC specimen was collected using a 22- or 23-gauge needle attached to a 10-mL syringe. At the time of specimen collection, clinical details were recorded through the patient's clinical records, and the gross appearance of the aspirate was noted. Three smears were prepared from each aspirate – first, fixed with ethanol for Papanicolaou (PAP) stain; second, stained with Romanowsky stain and were evaluated

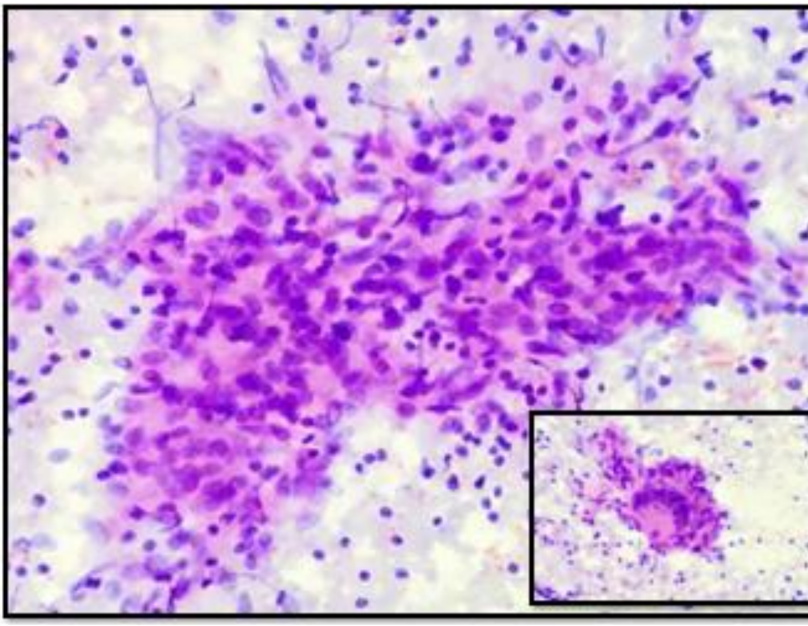

Figure 1. Smear shows epithelioid granuloma in lymphoid background; inset shows a Langhans type of multinucleated giant cell (PAP stain, x40).

for adequacy and examined; and third, stained by ZN stain for direct detection of acid-fast bacilli. *M. tuberculosis* appeared as a pink curve with slightly beaded rods. The cytological criteria for diagnosing tubercular lymphadenitis were the presence of granulomas with caseous necrosis (Figure 1) and the demonstration of AFB positivity (Figure 2).

The CBNAAT was performed using residual aspirated material. The material was mixed with buffer in ratio 1:2 in a pre-sterilized falcon tube and incubated at room temperature for 25 to 30 min. Two mL of this sample was then transferred to an Xpert cartridge using a Pasteur pipette, and the cartridge was loaded onto Xpert (Cepheid, Dx System Version 4.0c) machine. Results were reported as positive or negative for *M. tuberculosis*. CBNAAT gives a semi-quantitative estimate of the concentration of bacilli as defined by the cycle threshold range (very low – greater than 28; low – 22 to 28; medium – 16 to 22; high – less than 16) [1]. Rifampicin resistance results were reported as susceptible and resistant depending upon the presence of a mutation in the rpoB gene. Strains harboring mutations in the rpoB gene were resistant to Rifampicin.

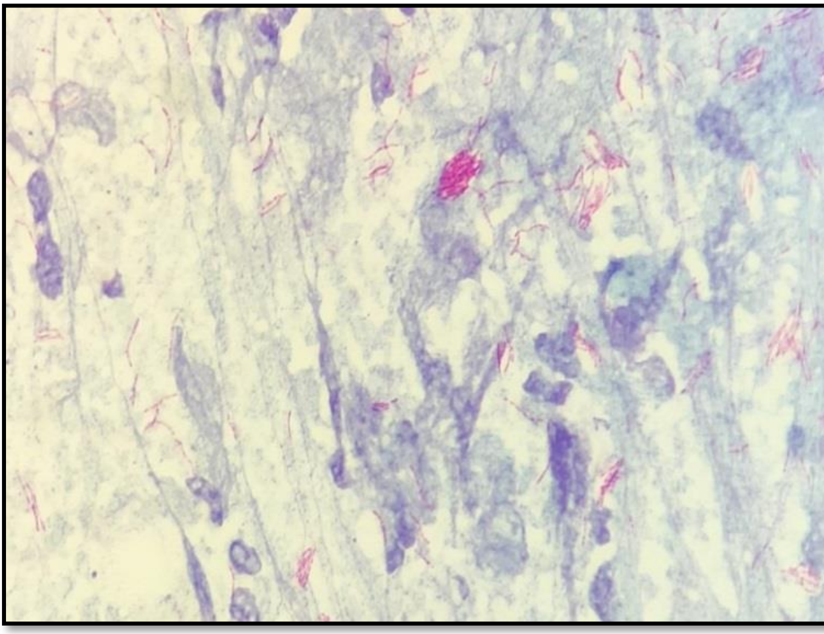

Figure 2. Smear shows acid-fast bacilli (ZN stain, x100).

2.4 Statistical Analysis

Data collected include age, gender, and site of lymphadenopathy. Sensitivity, specificity, positive predictive and negative predictive values of FNAC, CBNAAT and positivity on microscopy were calculated using standard formulae [7]:

$$Sensitivity = \frac{True\ Positive}{True\ Positive + False\ Negative}$$

$$Specificity = \frac{True\ Negative}{True\ Negative + False\ Positive}$$

$$Positive\ Predictive\ Value = \frac{Sensitivity \times Prevalence}{Sensitivity \times Prevalence + (1 - Specificity) \times (1 - Prevalence)}$$

$$Negative\ Predictive\ Value = \frac{Specificity \times (1 - Prevalence)}{Specificity \times (1 - Prevalence) + (1 - Sensitivity) \times Prevalence}$$

The data were analyzed using the SPSS 22.0 software (IBM, Chicago, IL, USA).

Table 1. Sensitivity, specificity, positive and negative predictive values of CBNAAT.

CBNAAT	Value (%)
Sensitivity	84.61%
Specificity	39.72%
Positive predictive value	93.54%
Negative predictive value	20.0%

Table 2. Age and sex distribution of total cases.

Age group	Number of cases		
	Male	Female	Total
1 month – 10 years	2	0	2
11 – 20 years	0	18	18
21 – 30 years	8	28	36
31 – 40 years	8	4	12
41 – 50 years	6	4	10
51 – 60 years	2	2	4
61 – 70 years	2	2	4
Total	28	58	86

3. Results

A total of 86 cases were studied. The age ranged from 3 to 65 years, with female predominance and the ratio being 1:2 (Table 2). Most cases were found in the age group 21-30 years, including the CBNAAT positive cases with a female preponderance (Table 3). The most common site was the right cervical lymph node (n=38 cases) (44.18%) (Table 4).

Granulomatous lymphadenitis was the most common cytological pattern (n=73 cases) (87.95%) on FNAC, followed by reactive lymphoid hyperplasia (n=5 cases) (5.81%) (Table 5). Cytomorphological features consistent with tuberculosis were seen in 87.95% of the cases. The CBNAAT was positive in 36.04% of the cases, and ZN stain microscopy was positive in 17.44% of cases (Table 6).

Out of 73 (87.95%) cases of tubercular lymphadenitis on FNAC, 29 cases (33.72%) were CBNAAT positive. The result of 46 cases (53.48%) did not correlate with FNA findings, 44 diagnosed with tuberculosis on FNAC were negative on the CBNAAT test, one case of reactive lymphoid hyperplasia, and one case of suppurative lymphadenitis on FNAC was positive on CBNAAT test (Table 6).

Out of 31 cases positive on the CBNAAT test, 15 cases were detected positive for AFB on microscopy, and all of them were diagnosed with tuberculosis on FNAC. Rifampicin resistance was found in 32.65% (10/31 positive cases) and 67.74% (21/31 positive cases) of the cases were sensitive to Rifampicin (Table 7).

Table 8 compares FNAC positives and negatives with CBNAAT positives and negatives.

Table 3. Age and sex distribution of CBNAAT positive cases.

Age group	Number of CBNAAT positive cases		
	Male	**Female**	**Total**
1 month – 10 years	0	0	0
11 – 20 years	0	8	8
21 – 30 years	2	13	15
31 – 40 years	4	0	4
41 – 50 years	2	2	4
51 – 60 years	0	0	0
61 – 70 years	0	0	0
Total	8	23	31

Table 4. Site distribution of cases.

Site	Number of cases (%)
Right cervical lymph node	38 (44.18)
Left cervical lymph node	28 (32.55)
Bilateral cervical lymph nodes	6 (6.97)
Right axillary lymph node	4 (4.65)
Right supraclavicular Lymph node	4 (4.65)
Left submandibular Lymph node	2 (2.32)
Left supraclavicular Lymph node	2 (2.32)
Left axillary lymph node	2 (2.32)
Total	86 (100.00)

Table 5. Cytological patterns on FNAC.

Pattern	Number of cases (%)
Granulomatous lymphadenitis	73 (87.95)
Reactive lymphoid hyperplasia	5 (5.81)
Metastatic carcinoma	3 (3.48)
Only hemorrhage	3 (3.48)
Suppurative lymphadenitis	2 (2.32)
Total	86 (100.00)

Table 6. Comparison of cytological findings with CBNAAT results and microscopy results.

Cytological pattern	Number of cases (%)	CBNAAT positive (%)	CBNAAT negative (%)	AFB Positive (%)	AFB Negative (%)
Granulomatous lymphadenitis	73 (87.95)	29 (33.72)	44 (51.16)	15 (17.44)	58 (67.44)
Reactive lymphoid hyperplasia	5 (5.81)	1 (1.16)	4 (4.65)	0 (0)	5 (5.81)
Suppurative lymphadenitis	2 (2.32)	1 (1.16)	1 (1.16)	0 (0)	2 (2.32)
Hemorrhagic	3 (3.48)	0 (0)	3 (3.48)	0 (0)	3 (3.48)
Metastatic carcinoma	3 (3.48)	0 (0)	3 (3.48)	0 (0)	3 (3.48)
Total	86 (100.00)	31 (36.04)	55 (63.95)	15 (17.44)	71 (82.55)

Table 7. CBNAAT results.

CBNAAT result	Number of cases (%)
Detected (resistant /L)	10 (32.25)
Detected (Sensitive /L)	19 (61.29)
Detected (Sensitive /H)	2 (6.45)
Total positive cases	31 (100.00)

Table 8. Comparison of FNAC positives and negatives with CBNAAT positives and negatives.

Test	FNAC positive	FNAC negative	Number of cases
CBNAAT positive	29	2	31
CBNAAT negative	44	11	55
Total	73	13	86

4. Discussion

This was a retrospective study based on the diagnosis of suspected tubercular lymphadenopathy by CBNAAT and smear microscopy compared to FNAC. The FNAC coupled with smear microscopy has been the investigation of choice in lymph node lesions. The cytological criteria for diagnosing tubercular lymphadenitis are the presence of granulomas with caseous necrosis and demonstration positivity on ZN staining [3]. Studies by Gomes et al. [8] and Das et al. [9] used the same diagnostic criteria.

In comparing age and gender-wise distribution of CBNAAT positive cases with other studies, it was found that the younger age group was affected more by female predominance, which correlated with other studies (Tables 9 and 10).

The most common site of lymphadenopathy in our study was cervical lymph nodes (76.74%), followed by axillary (6.97%) and supraclavicular (6.97%), which is in concordance with Khajuria et al. [15] and Chand et al. [16] studies.

In the present study, 46 cases of CBNAAT were non-concordant with FNAC, out of which 44 cases were diagnosed on FNA but were missed on CBNAAT, and two cases were negative on FNA and diagnosed on CBNAAT. Of the two FNA negative and CBNAAT positive cases, cytologically, one was a reactive lymph node, and the other was a suppurative lymphadenitis. In our study, the importance of CBNAAT was found in diagnosing tuberculosis in cases that were missed on FNAC and surely benefitted by CBNAAT.

In 44 FNA positive and CBNAAT negative cases, the bacterial load might be too low for the Gene Xpert to detect DNA of MTB complex [17]. Low bacillary load and its detection limit of 131 CFU/ml might be the reason for CBNAAT negativity in these patients [18].

As per the WHO guidelines, 44 FNA positive and CBNAAT negative cases received tuberculosis treatment, as they were positive on FNA and clinically suspicious [19]. Such CBNAAT negative cases may still have tuberculosis or Mycobacterium Other Than Tuberculosis (MOTT). In comparison of the sensitivity (39.72%) and specificity (84.61%) of CBNAAT in the present study, with Singh et al. (specificity = 90%,

Table 9. Comparison of age-wise distribution of CBNAAT positive cases with other studies.

Study	Age group	Percentage of cases
Present study	21-30	48.38
Yassin et al. [10]	15-24	30.7
Aroravk et al. [11]	15-24	38
Brayn et al. [12]	15-24	43
Mulualem et al. [13]	16-30	58

Table 10. Comparison of gender-wise distribution of CBNAAT positive cases with other studies.

Study	Male (%)	Female (%)
Present study	25.80	74.19
Brayn et al. [12]	46	54
Mulualem et al. [13]	67	76
Poojasingh et al. [14]	31	69

Table 11. Comparison of CBNAAT results with other studies.

Study	CBNAAT positivity (%)
Shakeel et al. [25]	36.3
Gour et al. [26]	40.0
Srwar et al. [27]	51.7
Moure et al. [28]	58.3
Anmol et al. [29]	62.7
Present study	36.04

sensitivity = 91%) and Ligthelm et al. (specificity = 88.9%, sensitivity = 96.7%), our study showed less sensitivity and more specificity [20, 21].

In the present study, out of 87.95% of the cases diagnosed as granulomatous lymphadenitis on FNAC, 39.72% were CBNAAT positive, and 20.54% were AFB positive. Overall, 17.44% of the cases showed AFB positivity, which correlates with the study done by Aggarwal et al. [22] and Nidhi et al. [23].

FNAC and AFB positivity are the preferred investigations of choice for diagnosis in cases of extra pulmonary tuberculosis. Still, they have some limitations, and CBNAAT was developed to overcome these limitations. CBNAAT identifies the targeted rpoB nucleic acid sequences and gives results in about 2 hours. No cross-reaction with other bacterial species was noted. It is a highly specific test as it uses five unique molecular probes and three specific primers to target rpoB gene for MTB [24]. It is a fully automated PCR-based test that detects DNA directly from the clinical specimens along with the detection of rifampicin resistance.

The present study showed 36.04% CBNAAT positivity which correlated with the study done by Shakeel et al. [25]. Various studies showed 36% to 96% CBNAAT positivity, as shown in Table 9. Technical causes like the second or third pass of FNA sent for CBNAAT and the scanty amount of material sent might be the reasons for low CBNAAT positivity [3].

5. Conclusion

FNAC is a cheap investigating modality and, when coupled with the demonstration of AFB, is the investigation of choice in suspected cases of tubercular lymphadenopathy. It gives a high level of accuracy and reduces morbidity and mortality of tuberculosis in resource-poor countries like India. However, CBNAAT has an important role in diagnosing tuberculosis as an adjuvant test using FNA material. In the present study, the sensitivity of CBNAAT was higher compared to microscopy. CBNAAT has the advantage of detecting Rifampicin resistance within a short time which is not possible with FNAC. The CBNAAT can detect cases missed on FNAC; hence, patients with positive CBNAAT results should be treated for tuberculosis.

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Conflict of Interest Statement

The author declares no conflict of interest.

Author Contributions: Research idea, data collection, data analysis and draft preparation, Diya Bajaj (D.B.); research idea, data collection, data analysis and review, M.K.G., P.T. and J.B.; research idea, data analysis and review, J.K.B. All authors have read and agreed to the published version of the manuscript.

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